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Research and design of a production system for a Tuberculosis (TB) sub-unit vaccine

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Introduction

Tuberculosis is an infectious disease caused by different strains of Mycobacterium Tuberculosis, and transmitted by respiratory fluids generated through coughing, sneezing, etc.

To this day, the only TB vaccine available is the BCG; however, it has various limitations. It is only effective on the young, and cannot be given to new-borns, who are at risk of infection. Additionally, as it is a live vaccine, an individual with a suppressed immune system cannot be vaccinated, e.g. HIV + individuals.

Purpose

Undergraduate research into a new Tuberculosis vaccine for those living in endemic parts of sub-Saharan Africa through the design of sub-unit vaccine process engineering and manufacturing systems. We intend to produce a subunit vaccine, containing antigenic proteins: RV0097 and FABG4.

Method Summary

Isolate the gene for proteins RV0097 and FABG4 from the TB bacteria.

Extract a plasmid DNA from a bacterium and cut it open with restriction enzymes. Insert the isolated protein-producing gene into the bacterial plasmid vector, forming recombinant DNA and introduce this to a yeast cell.

Grow the yeast cells in a bioreactor. The desired proteins are produced inside the yeast and need to be **isolated** via centrifugation and filtration.

Chromatography and other methods are required to **purify** our product. To increase stability, the vaccine must be **formulated**.

Design Specification

Temperature: 30-35°C

pH: 4-5 with the use of a phosphate buffer

Impeller speed: 600rpm

Media: YEPD (Yeast Extract Peptone Dextrose)

Sugar Concentration: 10%

Water grade: Grade 1 water certified by ACTM.

Mixing

Mixing of the bioreactor broth is needed for effective gas blending and to maintain optimum conditions throughout the solution, which are essential for the survival and growth of yeast. The mixing team designed a bioreactor system which ensured that these requirements were met in order to maximize yeast growth. A series of experiments were carried out to determine the dimensions and design of the bioreactor, its baffle system and impeller. The ideal stirrer speed was also calculated. The data obtained was used to construct a baffle system and to design the impellers and bioreactor. This bioreactor was used in a 24 hour trial run.

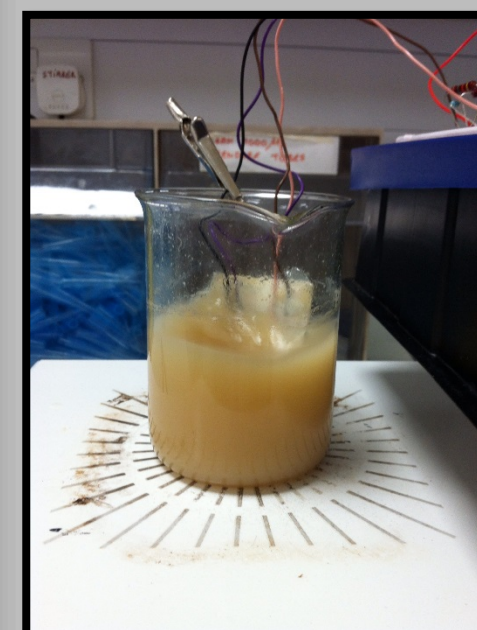
Medium Broth

The aim for this series of experiments was to determine the optimum conditions to maximize yeast growth. Various YEPD solutions of different sugar concentrations were prepared; initially a pH buffer was not added. A pre-cultured yeast inoculum was added to all the vessels and the fluid flow was turbulent at all times. At set time intervals, samples were taken from the fermentation broth and the absorbance recorded using a spectrophotometer. The results showed that the optimum sugar concentration for maximum yeast growth was 10% glucose, and the buffer was needed to maintain the pH.

Instrumentation

The aim for this series of experiments was to design a 4 electrode impedance probe that could be used to determine the optimum time at which the yeast should be collected. Experimentation of different electrode positions was carried out. Investigations into the AC frequency and the background solution enabled determination of when to remove the yeast. Unfortunately it quickly became clear that the electrical properties of the mixture were subject to many different conditions. Furthermore, the readings were low in precision; thus errors were found in the final results due to minute changes in conditions.

24 hr Run – Bioreactor results



Medium broth - The results from the 24 hour run showed that room temperature with a 10% glucose concentration was sufficient for the yeast inoculum to produce 0.06g per ml.

Instrumentation - The Experiment results were inconclusive. Ideally, another controlled experiment needs to be carried out.

Mixing Vessel Design:

Diameter of impeller: 40mm
Height of impeller from vessel bottom: 25mm
Impeller shape: Triangular Prism
Impeller Cross Section Side: 10mm
Vessel diameter: 65mm
Stirring power: 0.85W

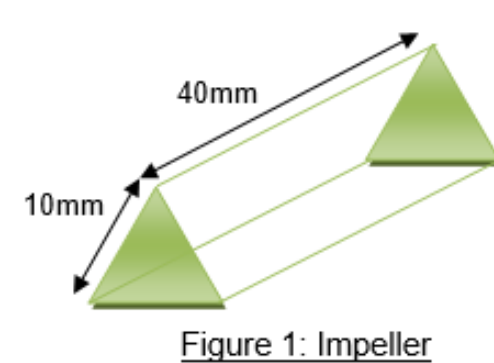


Figure 1: Impeller

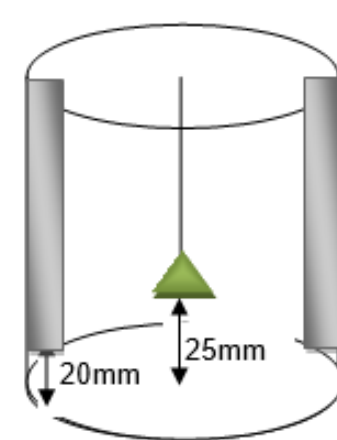


Figure 2: 250ml Beaker with Baffles (Bioreactor)

Note: The diagram is not to scale, and the cross-section is an equilateral triangle.

Key
■ Baffle
□ Beaker
■ Impeller

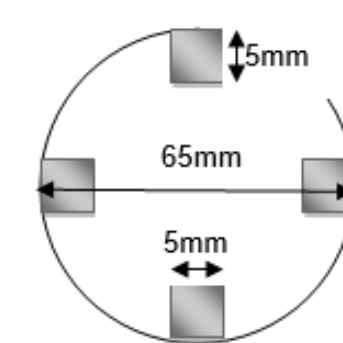


Figure 3: Bioreactor Cross-Section

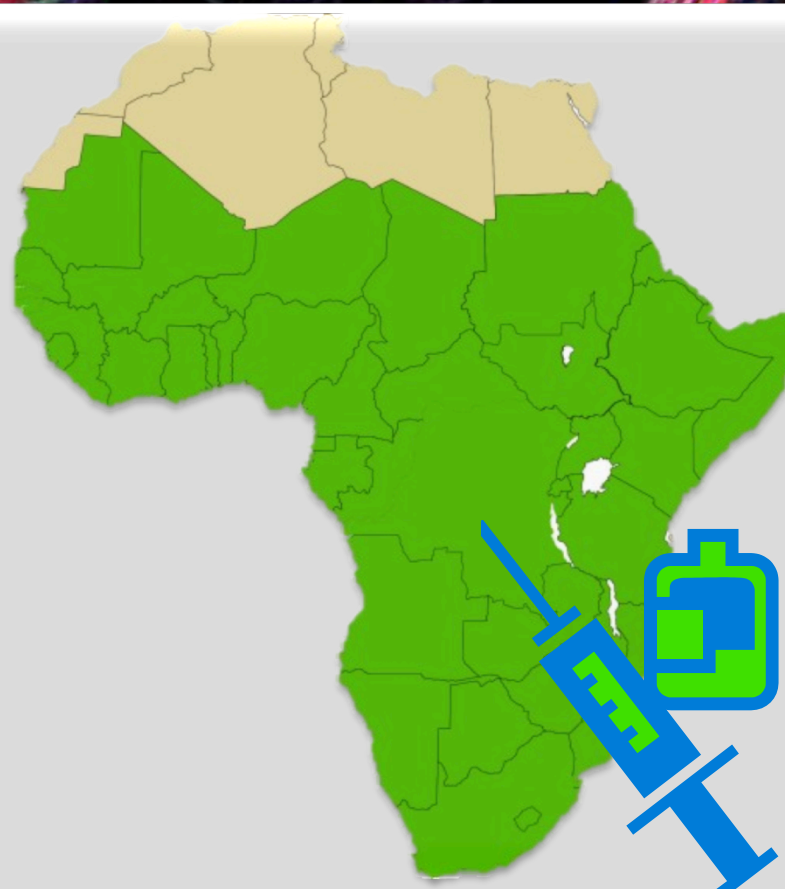
Marketing – Scale Up

Place: Sub-Saharan Africa

Target for 1st year of production:
33.6 million newborns

Vaccine Dose: 33.6 million shots
(15µg)

Budget: £50-150 million (based on
£1.50-4.50 per dose)



- [1] – <http://www.tradingeconomics.com/sub-saharan-africa/population-total-wb-data.html>
[2] – <http://www.rxlist.com/typhim-drug.htm>
[3] – <http://www.patient.co.uk/health/Hepatitis-B-Immunisation>
[4] – <http://www.ncbi.nlm.nih.gov/pubmed/9202817>

Scaling up the production of the vaccine to an industrial scale involved the use of results from the 24 hour run, and considering costs and efficiency also. 0.066 grams of wet cell weight per ml of solution were produced in the lab solution, and so it was assumed that this result could be extrapolated. Relevant figures were researched, including sub-Saharan Africa's birth rate (38.36 per 1000) [1], sub-Saharan Africa's population (874,841,049) [1], protein weight per vaccine dose (50 micrograms) [2], number of doses required per patient (three per patient) [3] and dose volume (0.25ml) [4]. It was determined that to produce three doses per patient for 33,558,900 new-borns in a year, with each dose containing 50 micrograms of proteins, the bioreactor would need to contain approximately 7,600 litres of broth. Allowing for space for foam formation and air, it was decided that a 10,000 litre bioreactor would be suitable. This would need to operate for 16 weeks to provide the required amount of proteins. The lab experiments were performed using a 250ml beaker with a height to diameter ratio of 1.159. Maintaining this ratio with a 10,000 litre bioreactor gave a diameter of 2.223m and a height of 2.576m. It was decided that the impeller type would be a standard six-blade Rushton turbine. It was calculated that the power consumption of the entire facility was 20kW, 15kW of which is required by the bioreactor control systems.